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A COMPARATIVE STUDY OF THE HARD CLAM GONAD DEVELOPMENTAL CYCLE[1]

RICHARD T. KECK, DON MAURER, AND HENRY LIND

University of Delaware, College of Marine Studies, Field Station, Lewes, Delaware 19958

The reproductive physiology of molluscs is of special interest due to their importance as food for man. A thorough knowledge of reproductive cycles is necessary for predicting annual recruitment, interpreting growth, mortality, and survival data, and in the mariculture of these species. A number of studies on the gonadal development of marine invertebrates have been conducted (Giese, 1959). Ansell (1963, 1964, 1968), Ansell and Loosmore (1963), Ansell, Lander, Coughlan, and Loosmore (1964), Ansell, Loosmore, and Lander, (1964), and Ansell and Lander (1967) performed extensive work on the reproduction, spawning, and growth of the hard clam, *Mercenaria mercenaria* (Linne), in England. Shaw (1964, 1965) and Ropes and Stickney (1965) characterized the gonadal cycle of the soft clam, *Mya arenaria* (Linne). Calabrese (1970) described the developmental cycle in the coot clam, *Mulinia lateralis* (Say), in Long Island Sound.

A study of the gonadal cycle of *Mercenaria mercenaria* was undertaken as a subproject of a comprehensive resource survey in Delaware Bay to determine if spawning was occurring and to what degree, to ascertain whether male and female cycles were synchronized, and to compare reproductive cycles of clams from different geographic regions as evidence for physiological races. Loosanoff (1937a) and Porter (1964) studied the gonadal cycles of hard clams in Long Island Sound and North Carolina, respectively. Environmental differences produced different physiological responses in respect to timing of development and developmental pattern. Porter (1964) suggested that the differences were a possible expression of physiological races caused by phenotypic response to environmental factors. This phenomenon is well known in marine organisms and is particularly well documented in the American oyster (Galtsoff, 1964).

Materials and Methods

Twenty hard clams were collected monthly for 34 months (January 1971 through October 1973) from each of two areas in Delaware Bay. Area 1, referred to as Delaware Bay, is located approximately 2.7 km northeast of the mouth of Roosevelt Inlet (Coast and Geodetic Survey 1218). The area is characterized by a soft mud (40–80% silt-clay) bottom and is approximately 3–4.5 m deep at mean low water. The clams in this area were obtained by dredging with a standard oyster scrape. Area 2, the Henlopen intertidal flat, is located near the mouth of Delaware Bay inside Cape Henlopen. The area is characterized by a hard sand bottom (5–10% silt-clay). These clams were obtained by hand-raking during periods of low tide. All clams were returned to the laboratory and stored dry in

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a cool room at approximately 15° C. In all cases tissue samples were removed within 24 hours of the collection in the field.

Water temperatures in these areas are influenced by both season and tide. During the summer months, temperature falls on the flood tide and rises on the ebb tide. This condition is reversed in winter when the average change during each tidal cycle is approximately 3° C (DeWitt, 1968). Mean monthly water temperatures for lower Delaware Bay with ranges excluding those extremes that occur in less than 1% of the data compiled from Brower, Sisk, and Quayle (1972) appear in Figure 1. Temperature data from S. G. Landers (personal

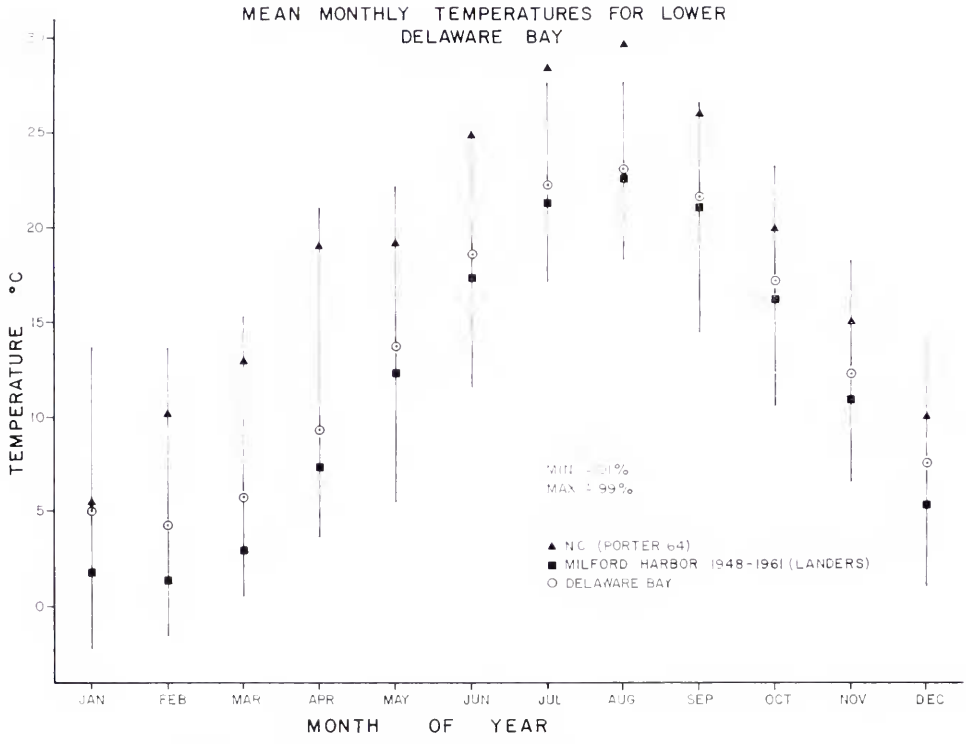


FIGURE 1. Mean monthly temperatures for lower Delaware Bay, North Carolina, and Long Island Sound.

communication, National Marine Fisheries Service Laboratory, Milford, Connecticut) and Porter (1946) are included in Figure 1 to contrast the different physical regimes in the areas for which gonadal data are compared later in the text.

A 1 cm^2 sample of mantle, gonad, and underlying digestive gland was removed from each of the 20 clams and placed in Bouin's fixative for at least 48 hours. The tissue samples were removed from the area located at the midpoint of a horizontal line connecting the anterior and posterior adductor muscles. Tissues were then prepared for cutting by dehydration in alcohol, clearing in xylene, and embedding in paraffin (Humason, 1967). Gonadal tissue was sectioned at 10 μm

and stained with Harris' or Delafield's hematoxylin and counter-stained with eosin Y. Sections were examined under a light microscope and classified by the developmental stage.

To quantify the stage of development of the gonads, the size and number of ova in 20 random sections of tissue 0.2 mm^2 on each female slide were measured and counted. Although the size of an egg depends on the level at which it is cut, the average size based on large numbers of measurements accurately indicates stage of development. The point area method was used to analyze male slides (Ivantsch, 1970). Male gonads were projected onto graph paper and traced to determine the percentage of the lumen filled with radiating bands of spermatids or spermatozoa.

Photomicrographs of representative stages of gametogenesis were taken with a light microscope at × 100 magnification using a standard camera adapter and Nikormat camera. A fine grain, high contrast film, Panatomic X, ASA32, was used.

Results

Developmental stages of the male

The following description of the male and female developmental stages is based upon our observations and criteria compiled from Ropes and Stickney (1965), Porter (1964), and Loosanoff (1937a). The individual stages define qualitative criteria describing the continuous process of change occurring in cells and tissues during the maturation of gonad. Because the terminology for the indifferent phase presents semantic problems, a brief definition for both male and female stages is as follows: although the term indifferent has been applied to specimens that could not be sexually differentiated, it also implies low levels of either spermiogenic or ovogenic acitivity with correspondingly low numbers of recognizable sex cells. The authors found no specimens where it was impossible to determine sex. Basic follicular differences appear to allow differentiation between sexes without the presence of mature sex cells. However, because inactive implies a static condition where absolutely no morphological or biochemical activity is proceeding, the term indifferent appears to be more biologically appropriate to describe that stage occurring between spawned and onset of new development.

Indifferent or inactive stage. The follicle was usually expanded, and only rarely compressed. The basal membrane and follicular cells are dominant and follicular cells often filled the lumen surrounding pycnotic or aberrant cells. The presence of these cells may indicate various forms of structural disintegration. The nuclear boundary is obliterated and the chromatin contracts into deeply stained irregular masses. The remainder of the cell may lose its staining capacity. When the follicle was expanded, there were few, if any, spermatogonia or spermatocytes at the periphery of the lumen. In some cases, the expanded lumen contained spermatozoa in random arrangement and of varying density (Fig. 2A).

Developing or active stage. This phase included the entire process of spermatogenesis. The follicle in this and succeeding stages was expanded. The basal membrane and its attached follicular cells become less apparent as development proceeds. Numerous spermatogonia were present in early stages near the periphery of the lumen. As development proceeded, spermatocytes and spermatids were

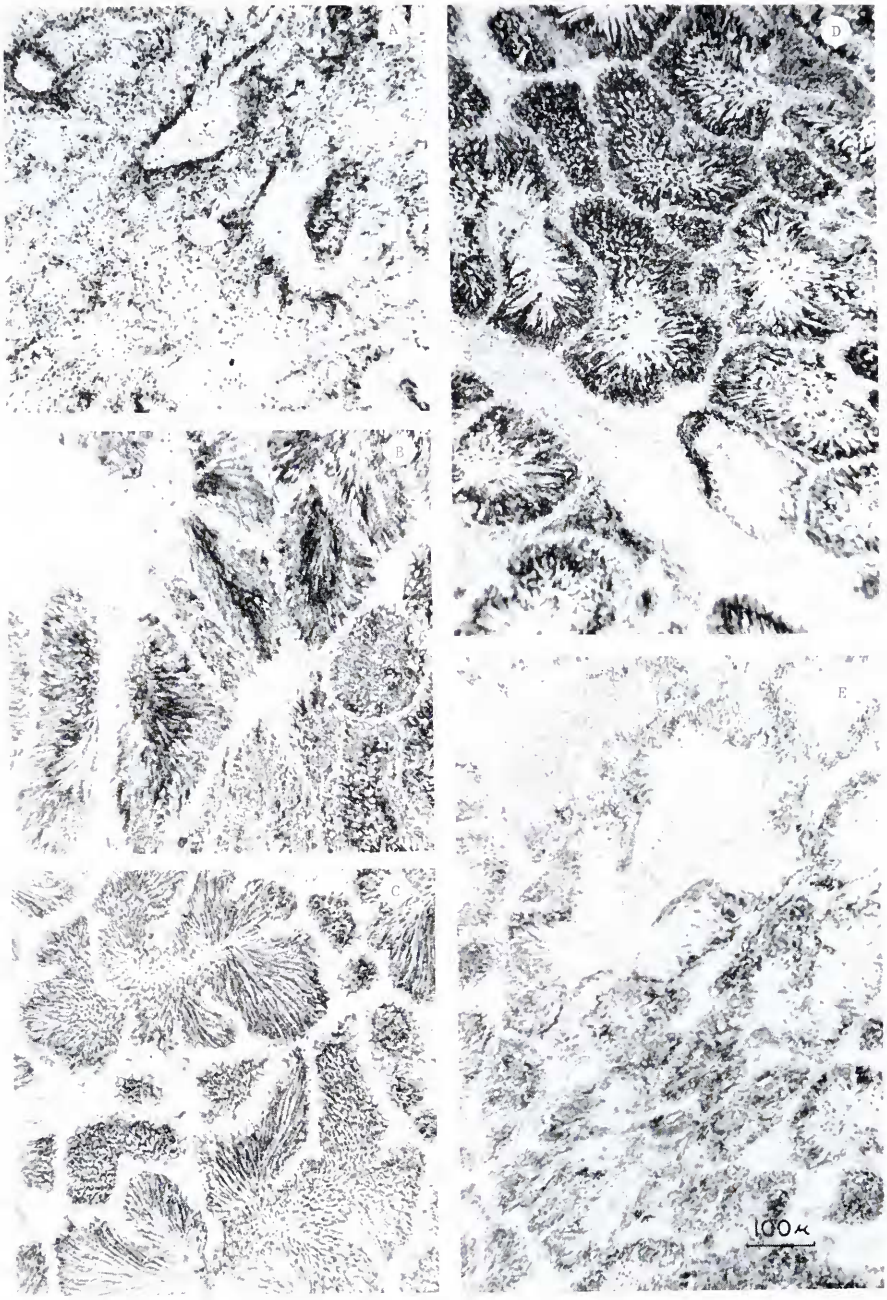


FIGURE 2. Male hard clam developmental stages: (A) indifferent; (B) developing; (C) ripe; (D) partially spawned; (E) totally spawned.

predominant making it difficult to see follicular cells or spermatogonia. Toward the center of the lumen, spermatids differentiated into spermatozoa arranged in dense radiating bands (Fig. 2B).

Ripe stage. The lumen was filled with dense radiating bands of spermatozoa. In fully ripe individuals approximately 95% of the lumen was filled with spermatozoa. The spermatocyte and spermatid layers were thinner than in the developing stage. The spermatid band was usually thicker than the spermatocyte band. However, in extremely ripe individuals the spermatid band was difficult to see due to staining similarities of spermatids and spermatozoa and the overwhelming abundance of mature spermatozoa (Fig. 2C).

Spawned stage. In this stage, consisting of a partially spawned or totally spawned or spent state, the spermatogenic layer (spermatocytes and spermatids) was extremely thin or nonexistent. The lumen had fewer spermatozoa than the ripe stage. The remaining spermatozoa remained in radiating bands. In totally spawned individuals the empty lumen contained few sex cells. The remaining spermatozoa were found near the edge of the follicle, and different amounts of spermatozoa were randomly arranged in the center of the lumen (Figs. 2D and 2E).

Developmental stages of the female

Indifferent or inactive stage. Lumina were semi-contracted and contained unspawned ovocytes. Extremely small ovocytes were still embedded in the follicle wall and recognizable due to the staining of the large basophilic nucleus. This stage was practically nonexistent in the female cycle because partially spawned or totally spawned individuals start ovogenic activity immediately (Fig. 3E).

Developing or active stage. The developing stage was characterized by an increase in the number of ovocytes and size of existing ovocytes. The degree of development was determined by counting and measuring ovocytes. During early development, the ovocytes ranged in diameter between 20–30 μm; as maturation approached, ovocytes varied between 40–50 μm in diameter. The developmental stage was also characterized by thick follicular walls and cytoplasmic inclusions which may be nutritive in function. Small follicles, especially near the periphery of the gonadal mass, frequently were filled with follicular cells. Although in later stages of development many mature or ripe ovocytes existed, there was continuing ovogenic avtivity as shown by the presence of small ovocytes near the periphery of the follicle. Partition cells were often seen during this stage and were also commonly found near the edges of the gonadal mass (Figs. 3A, 3B, and 3D).

Ripe stage. The ripe stage, characterized by large numbers of large mature ovocytes which were more numerous than less developed ovocytes, were generally between 50 and 60 μm in diameter. The follicular wall was extremely thin and expanded and ovogenic activity had practically ceased. The basal attachment of the mature ovocytes was less evident and most ovocytes appeared to be free within the follicular lumen (Fig. 3C).

Spawned stage. The spawned stage consisted of two substages, partially and totally spawned. Totally spawned females were rare. The lumen of the ovarian follicle generally contained a few ripe ovocytes. The follicular wall was semi-

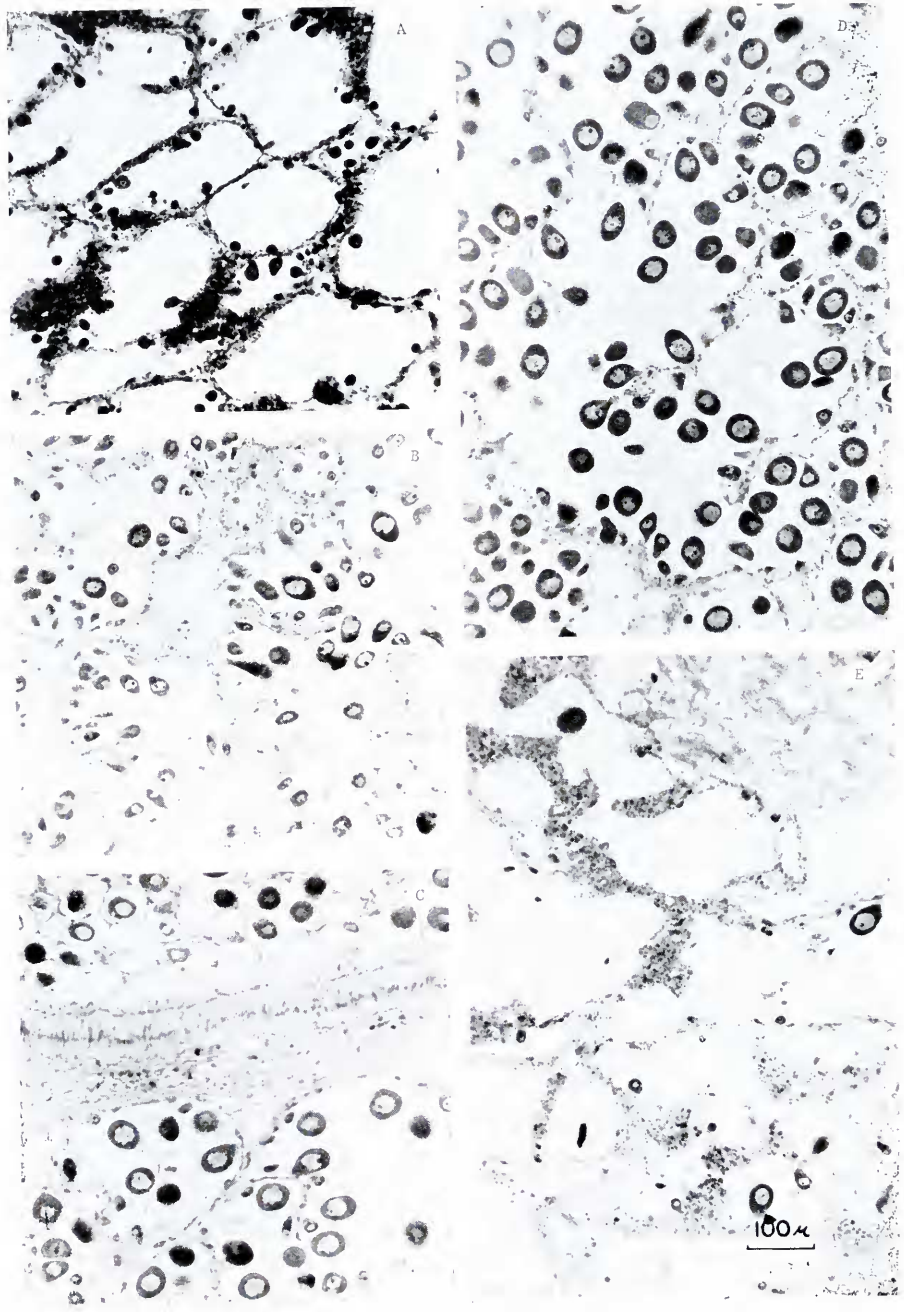


FIGURE 3. Female hard clam developmental stages: (A) developing; (B) developing; (C) partition cells; (D) ripe; (E) spawned, indifferent.

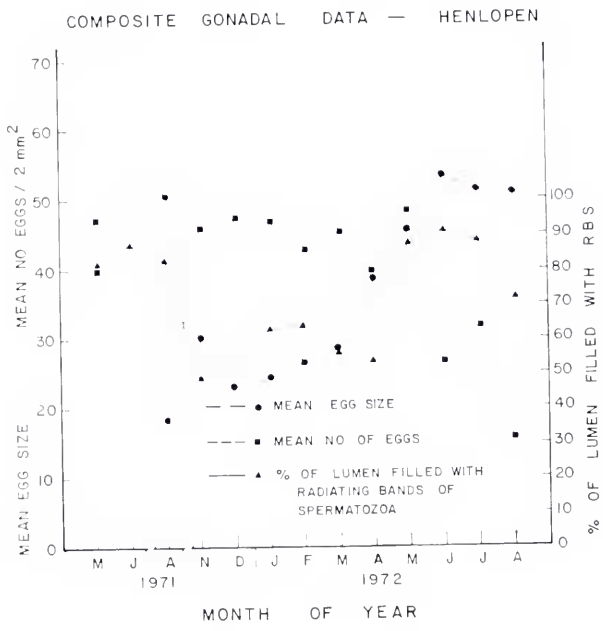
contracted, and thickening may have begun. Ovogenic activity was low, but evidence of regeneration was usually apparent in spawned individuals (Fig. 3E).

Seasonal variation of the gonadal cycle

Quantitative data describing the male and female developmental cycles appear in Figures 4 and 5. The male and female cycles were in phase for both Delaware Bay and Henlopen clams. The percentage of lumina filled with radiating bands of spermatozoa and egg size of females coincided. Peak spawning occurred in the month of August. In the male, peak spawning activity was marked by the beginning of a precipitous decline in percentage of lumina filled with radiating bands of spermatozoa. In the female, heavy spawning activity was represented by the greatest difference between egg size (large) and egg number (low). Delaware Bay clams showed that increases in egg size and egg number were parallel until May. After May, these data diverged, indicating that partial spawning occurred from June to October. The egg number (data) for Delaware Bay clams showed that regeneration started in early August and that ovogenic activity continued slowly toward maturity in May. Henlopen clams showed a sinusoidal pattern in which egg size and egg number shifted. This pattern appears natural in that large egg size indicates there should be fewer eggs per given unit of tissue and small egg size denotes the converse. This phenomenon occurs as the eggs ripen and the follicular lumina expand. The shift usually occurred in May and October, which again reflects the length of the spawning season. The shift also indicated a more rapid regeneration. The Henlopen clams produced the majority of their new ovocytes between September and December. From December to May the egg number was relatively constant with a gradual increase in size reflecting maturation. Comparison of egg number per unit area for Figures 4 and 5 showed that Henlopen clams produced more eggs per unit area, while Delaware Bay clams generally produced larger eggs, representing a possible difference in reproductive potential.

A three-year composite of qualitative observations reflecting all developmental data collected per month for January 1971 through October 1973 is presented in Figures 6 and 7. Data are represented as percentage of individuals in each developmental stage per month. The data show that there was no significant difference in developmental pattern between Delaware Bay and Henlopen clams. However, males from both Delaware Bay and Henlopen seemed to lag behind females until late spring when rapid spermatogenic activity brought the male and female cycles into phase. The lag is probably a result of the fact that many male clams spend a period of time in the indifferent or the inactive stage (fall and winter months), while the females start regeneration immediately.

The qualitative and quantitative data support a similar developmental trend. Spawning activity commenced in June and continued until October where some individual appeared totally spent. Peak spawning activity for both males and females occurred in August and September. Females started ovogenic activity immediately, as early as July and August for partial spawners. A significant percentage of the males became indifferent after spawning in October (Henlopen mean, 40% ; Delaware Bay mean, 18%). This percentage of indifferent males remained relatively constant until May when rapid development occurred.



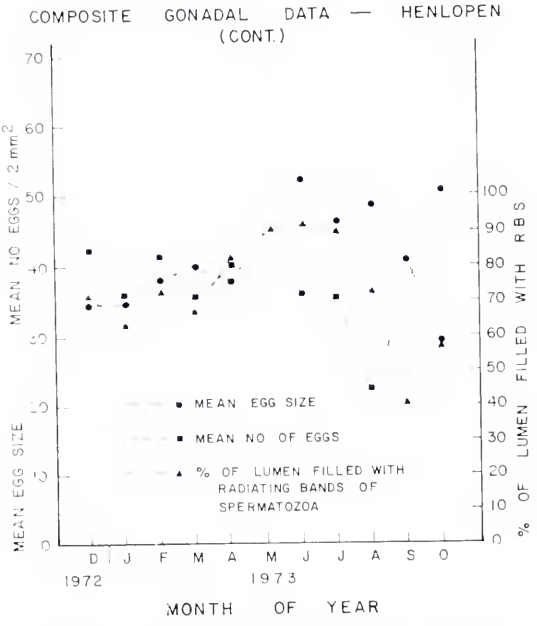


FIGURE 4. Quantitative gonadal data, Henlopen.

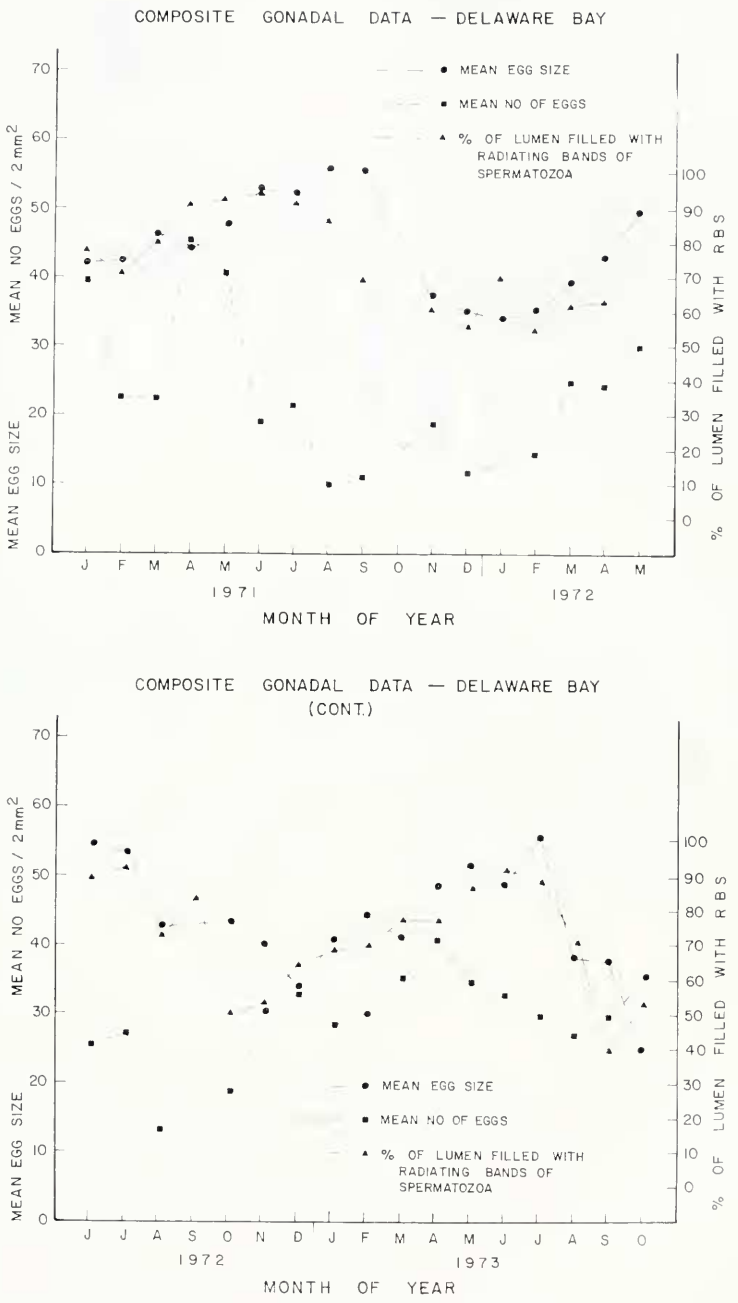


FIGURE 5. Quantitative gonadal data, Delaware Bay.

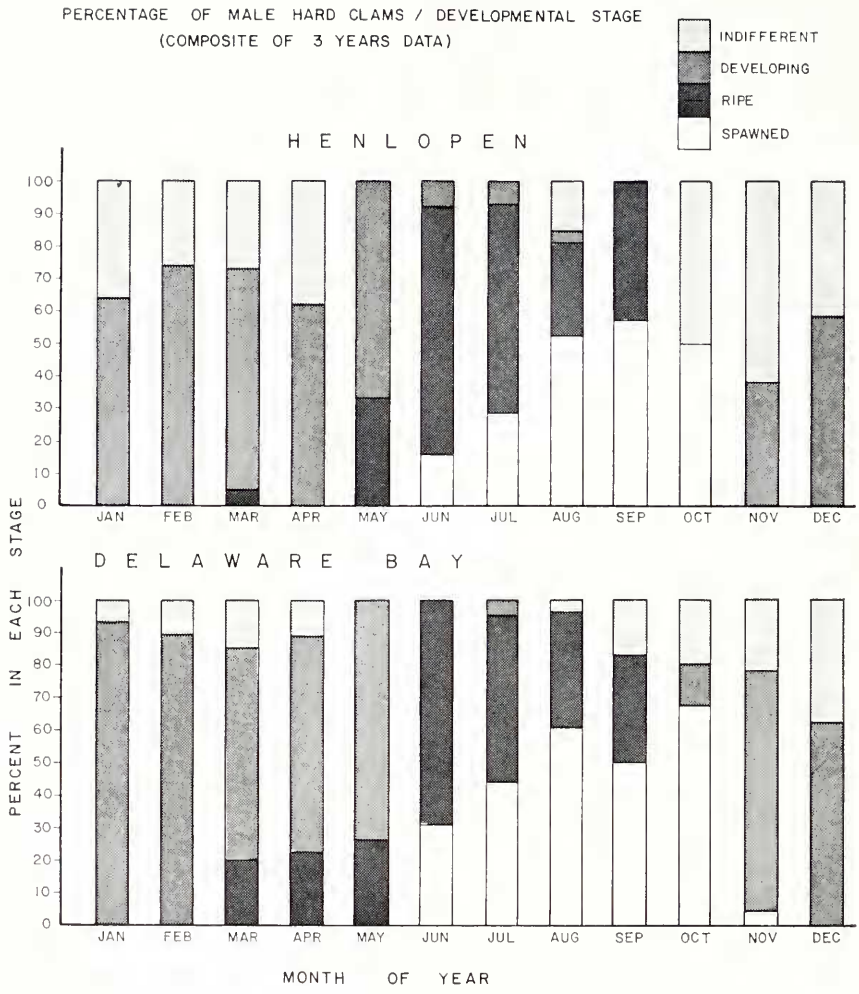


FIGURE 6. Percentage of male hard clams/developmental stage (qualitative criteria).

Analysis of the female developing stage requires consideration of both qualitative and quantitative data. Developmental stages in Henlopen and Delaware Bay females indicated a similar pattern. However, although both groups were in the developing stage, the mechanism was different. Henlopen clams regenerated large numbers of ovocytes rapidly, which slowly increased in size during the winter and spring. Delaware Bay clams developed slowly in both size and number of ova for a long period of time. Ripe individuals appeared as early as December (10% of individuals). From January through April, there was a slight increase in the percentage of ripe individuals per month. Between April and May, rapid ovogenic activity produced large numbers of ripe individuals. June was characterized by the greatest percentage of ripe individuals and the onset of spawning activity for both males and females.

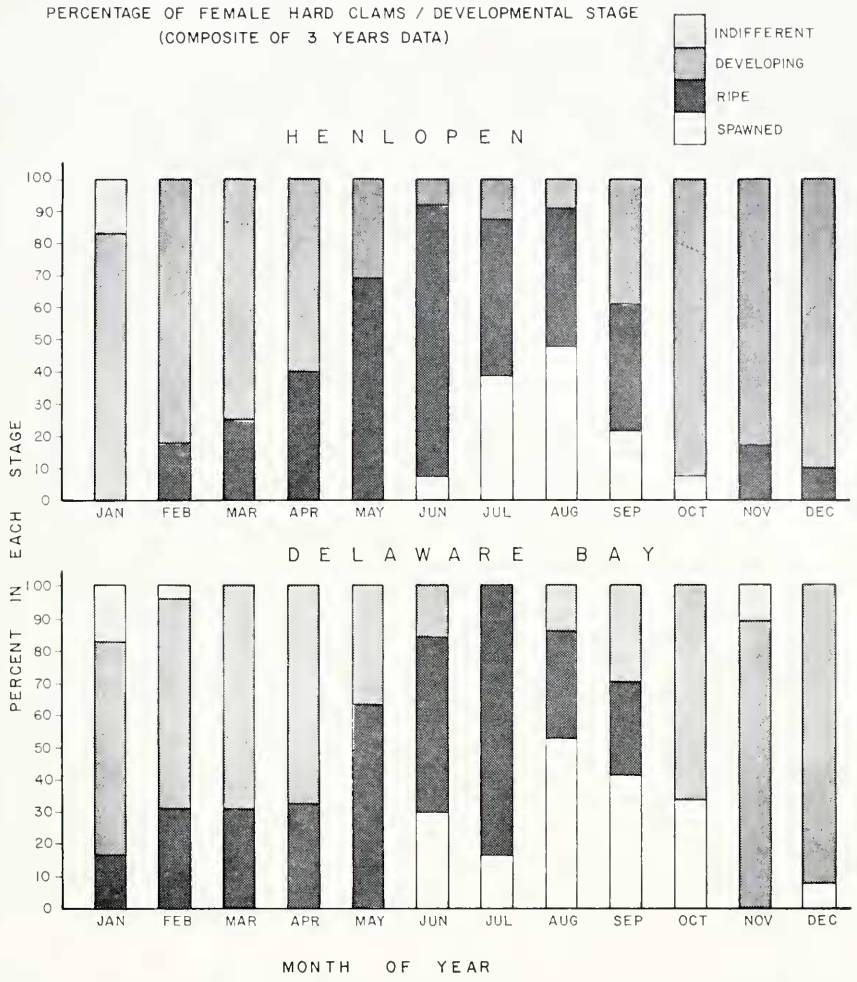


FIGURE 7. Percentage of female hard clams/developmental stage (qualitative criteria).

DISCUSSION

The data presented here provide some similarities and differences to the work of Loosanoff (1937a) and Porter (1964). Temperature is an important factor affecting the regulation of the gonadal cycle in a variety of marine bivalves (Galtsoff, 1964; Loosanoff, 1937a, 1937b; Landers, 1954; Carriker, 1961; Ansell, Lander, Coughlan, and Loosmore, 1964; Calabrese, 1970; Giese, 1959). The temperature and salinity regimes which exist in Long Island Sound, Delaware, and North Carolina are different. However, mean water temperatures in Delaware more closely resemble mean temperatures in Long Island Sound than those in North Carolina (Fig. 1). Local conditions can drastically affect the gonadal cycle. Ansell, Lander, Coughlan, and Loosmore (1964) and Tinsman (1973)

have shown that thermal effluent produced premature ripening of gonads in hard clams and oysters, respectively. Carriker (1961) showed that depth of water and circulation together with temperature greatly affect the onset of spawning activity in hard clams. H. G. Lind (personal communication, University of Delaware) showed that temperatures in a shallow Massachusetts salt pond caused two spawning periods in the soft clam, *Mya arenaria*. There are normally two spawning periods south and one north of Cape Cod for soft clams in estuarine waters (Ropes and Stickney, 1965). Landers (1954) suggested that intertidal clams spawned earlier than those from deeper water. Keck, Maurer, Daisey, and Sterling (1973) reported this phenomenon for oysters in tributaries of the Delaware Bay.

Table I provides a comparison of monthly developmental differences among bivalves studied by Loosanoff (1937a), Porter (1964), and those under present investigation. The spawning period, June to October, was similar to that reported by Porter (1964) and the present study. Loosanoff noted that spawning did not occur until late July in Long Island waters. Spawning in males was more complete than in females in all three areas. The proportion of totally spawned individuals of both sexes increased from south to north. R. W. Menzel (personal communication, Florida State University) states that field observations indicated that *M. mercenaria* and *M. campechiensis* complete spawning as early as April in Florida waters and are much more difficult to condition than northern clams. This appears to be linked to the greater variability of summer temperatures in northern waters. The rate of temperature change probably provides a stronger spawning stimulus than absolute temperature. Normal spawning temperatures are slightly different in the three regions (27–30° C in North Carolina, Porter, 1964; 25–27° C in Delaware, and 23–25° C in Long Island, Loosanoff, 1937a). Regeneration of gonadal tissue was evident as early as June in North Carolina waters where Porter observed that a "late ripeness" and capability to respawn were evident. Although regeneration was evident in August in Delaware bivalves, the restoration process required a longer refractory period. Clams that showed a high degree of ovogenic activity after partial spawning were those that appeared ripe in late fall and early winter. Loosanoff (1937a) reported that regeneration also starts immediately after spawning in Long Island waters, that is, during September and October.

Extrusion of unspent ova remains an unexplained phenomenon in this study. Loosanoff (1937a, 1937b) reported that the majority of unspent ova were extruded in a normal manner by November. Porter (1964) reported that at least 50% of his clams retained a ripe appearance through the fall and winter. Major extrusion occurred during February or March. In the present study, there was insufficient information to determine the time of extrusion. However, throughout the late fall, winter, and springs, partition cells appeared near the periphery of the gonadal mass in mature individuals. Porter (1964) suggested that cytological destruction may occur within the partition cells. Quite possibly, as suggested by Ansell (1961), the older ovocytes were carried over in partition cells and redeveloped follicles. As noted by Loosanoff (1937a), the more complete the spawning, the more rapid regeneration begins.

The pattern and timing of development differ widely among clams from different geographic areas. Porter (1964) reported major primary redevelopment in

TABLE I

Comparison of the sequence of hard clam gonadal development in three geographic regions

Comparative developmental stage	North Carolina (Porter, 1964)	Delaware (present study)	Long Island (Loosanoff, 1937a)
Partial spawning ♀ + ♂	June	June	July
Maximum spawning	Aug. (27–30° C)	Aug. (25–27° C)	Aug. (23–25° C)
Length of spawning season ♀ + ♂	June–Sept.	June–Sept.	July–Aug.
Regeneration ♀	[early (summer) or late (winter)]	Oct.–Dec.	Oct.–Dec.
Percent remaining ripe + timing of extrusion	50% through Dec.	Fate of unspent ova undetermined; partition cells—evident Nov.–March	Extrusion complete by Nov.
Percent of individuals in an indifferent stage ♀ + ♂	60–90%	Approximately 40% of males—less than 10% females	No indifferent stage
Major period of development ♀	Dec.–Jan.	Long, slow process; Oct.–March	Long, slow process; Oct.–March
Major period of rapid gametogenic activity ♀ + ♂	March	April	May
Presence of follicular cells	Present in developing stages	Prominent in developing stages	Absent
Majority of individuals in ripe stage ♀ + ♂	May	May and June	June

December and January with secondary activity in March. Loosanoff (1937a, 1937b) described rapid redevelopment in October through November. December through April marks a slowed period of continued maturation.

The present study provided two divergent patterns of development. The Henlopen clams developed very similarly to those discussed by Loosanoff (1937a), but preceding that timetable by approximately one month. The Delaware Bay clams provided a pattern different from that reported by both Porter and Loosanoff. In 1971–1972 (Fig. 4), female redevelopment started late in November and then continued with a slow increase in size and number of eggs. In 1972–1973, the process started earlier, but the slow steadily increasing trend was again apparent. Henlopen clams are in close proximity to cooler oceanic water in the summer and warmer oceanic water in the fall and winter, and this may be responsible for the difference in developmental pattern. Male clams from both Delaware Bay and Henlopen developed similarly to those discussed by Porter. However, a smaller

percentage of clams in Delaware waters were found in the indifferent stage. Loosanoff did not report any indifferent stage for Long Island male clams. Vigorous spermatogenic activity occurred in March (North Carolina), May (Delaware), and June (Long Island). In addition, Long Island clams showed more advanced spermatogenic activity in the fall than either Delaware or North Carolina clams.

The presence of follicular cells of North Carolina clams and their absence in Long Island clams was a major difference noted by Porter (1964). Both Delaware and Henlopen clams contained follicular cells. These vacuolated cells, as reported by Porter (1964), appeared in small follicles near the periphery of the gonadal mass. These cells were evident in all tissues categorized as developing (fall through spring). Follicular cells were reported by Ansell (1961) in *Venus striatula* and Ropes and Stickney (1962) in *Mya arenaria*. Various functions such as nutrition or phagocytosis have been ascribed to the cells. Because of their location in small follicles, it is likely they have a function in expansion of the developing follicle.

The study of the hard clam gonadal cycle in Delaware Bay provides data from an intermediate location to compare with Loosanoff (1937a) and Porter (1964). The data collected by Loosanoff and Porter had the advantage of large number of individuals during a one-year period. The present study had fewer samples/year, but the data were collected over a three-year period and provide almost complete coverage through several cycles.

The clams in Delaware Bay show gonadal development intermediate between Long Island and North Carolina clams. Also, clams from essentially the same area (Delaware Bay and Henlopen tidal flats) displayed divergent developmental patterns. These different gonadal patterns are due to environmental differences. Loosanoff commented that the sexual cycle of the hard clam was not in phase with other bivalve molluscs. The major period of development was in the fall as water temperatures decreased. Recent unpublished studies by the authors suggest that the glycogen cycle for the clam is widely different from that of the oyster. A spent oyster is devoid of sex products and generally in poor condition. The hard clam retains a relatively high proportion of glycogen or condition index even after spawning. The maintenance of a high condition level allows immediate gonadal redevelopment in the fall.

Summary

1. This study has shown that hard clam reproductive cycles in Delaware Bay are in phase and that spawning activity during 1971–1973 was of sufficient intensity to provide ample larval stocks.

2. The gonad developmental patterns for clams in Delaware are intermediate between those for Long Island and North Carolina. The data provide supporting evidence that different physiological races exist in the three areas compared. A further test on the validity of physiological races could be determined by studying the developmental patterns of Long Island and North Carolina clams held experimentally in Delaware Bay.

3. Environmental factors attribute to subtle differences in reproductive physiology as evidenced by the different mechanisms of regeneration and development between Delaware Bay and Henlopen hard clam females.

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